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# DATA EVALUATION RECORD

**DP BARCODE:** D176790
**MRID:** MRID 414434-07

**STUDY TYPE:** Freshwater aquatic invertebrate toxicity and pathogenicity testing: Tier I, 154A-20, Subdivision M.

**STUDY TITLE:** Young, B. M. 1990. 21-day prolonged static renewal toxicity of Dipel technical to *Daphnia magna*. Analytical Bio-Chemistry Laboratories, Inc. Aquatic Toxicology Division. Columbia, MO. ABC Laboratory report #38417. Submitted by Abbott Laboratories. North Chicago, IL.

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**PEER REVIEW BY:**

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**REVIEW CONCLUSIONS:** This study provides supplemental information. It does not satisfy 154A-20 core requirements because a sterile culture filtrate control [Section (b)(3)(ii) 154A-20 Subdivision M] was not tested, nor were a sufficient number of treatment concentrations tested within a dosage-mortality response range to allow the determination of an accurate $LC_{50}$ and 95% confidence limits [Sections (b)(7)(i) and (c)(1) 154A-20 Subdivision M]. Since 50 ppm Dipel killed 100% of the test daphnids, and 5 ppm killed 2.5%, the $LC_{50}$ lies between those two concentrations. Thus, Dipel is either slightly or moderately toxic to *D. magna*. The no observed effect level (NOEL) is <5 ppm, and the lowest observed effect level (LOEL) is 5 ppm.

**ADEQUACY OF STUDY:** Supplemental.

**RECOMMENDATIONS:** EFED recommends that the registrant repeat the toxicity tests using a graded series of dilutions (minimum of five, preferably seven) where about 0-5%, and 90-100% mortality, is expected at the lowest and highest dosages, respectively. $LC_{50}$ values and corresponding 95% confidence limits should be

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calculated using the resulting dosage mortality data. Test materials should be Dipel technical powder, a heat attenuated Dipel technical powder control, and a filter sterilized fermentation broth control to determine if the mortality observed in the current test was due to turbidity, the *B. thuringiensis* subsp. *kurstaki* spore-crystal complex, or unintended exotoxins. These controls should be at rates representative of the top treatment level.

The test suspensions should be aerated to keep the technical powder in suspension, and the level of technical powder in the test suspensions should be verified during the test by determining *B. thuringiensis* spore counts at appropriate intervals. $LC_{50}$ values and corresponding 95% confidence limits, based on actual technical material levels - not nominal, should be calculated using the resulting dosage mortality data.

**MATERIALS AND METHODS**: First instar daphnids were exposed to Dipel technical powder nominal concentrations of 0, 5, 50, and 100 mg/l (0, 5, 50, and 100 ppm) for 21 days. A sterile culture filtrate or attenuated Dipel technical powder control was not used. Forty daphnids were exposed to each concentration. Four replications of 10 daphnids each were used. Each replication was held in 1 liter glass jars containing 400 ml of suspension. The jars were held at 19-21°C, and the suspensions in each jar were continuously aerated. The jars were illuminated with cool-white fluorescent lights at an intensity of 40-80 footcandles, and a photoperiod of 16L:8D.

Daphnids were fed *Selenastrum capricornutum* alga, Tetramin®, cereal leaf, and yeast chow suspension at least twice daily. Mortalities, abnormal effects, and time to first brood determined daily. Reproductive success determined by counting and discarding offspring every Tuesday, Thursday, and Sunday.

Daphnia survival, growth, and reproductive data were analyzed by analysis of variance and Dunnetts's multiple means comparison test to determine if significant differences existed. The estimated $LC_{50}$ was calculated using a program developed by Stephan et al.

**REPORTED RESULTS:**

Table 1. Mean survival, reproduction, and length of daphnids exposed for 21 days to several concentrations of Dipel.

| Nominal conc. (mg/l) | % Survival | Length (mm) | Time to first brood (days) | Progeny/day |
|---|---|---|---|---|
| 0 (control) | 100 | 4.0 | 8.0 | 7.5 |
| 5 | 98 | 3.5* | 9.0 | 2.5* |
| 50 | 0 | - | - | - |
| 100 | 0 | - | - | - |

* Significantly different from control at P °0.05.

Table 2. No observed effect level (NOEL) and lowest observed effect level (LOEL) from toxicity test, in mg/l, with daphnids and Dipel.

| Biological Endpoint | NOEL | LOEL |
|---|---|---|
| First generation survival | 5 | <50 |
| Time to first brood | 5 | >5 |
| Number of young produced | <5 | 5 |
| Length of survivors | <5 | 5 |

The estimated $EC_{50}$ was 14 ppm (there were not enough data points to calculate a valid value).

**STUDY AUTHOR'S CONCLUSIONS:** "Daphnid reproduction and growth appear to be significantly affected at Dipel Technical concentrations of 5, 50 and 100 mg/l. Daphnid survival and time to first brood were not significantly affected by Dipel Technical at 5 mg/l. Therefore, the MATC and no-effect concentration (NOEC) were estimated to be <5 mg/l after 21 days. These results may not be attributed to the active ingredient of Dipel Technical but could be attributed to the quantity of solids present. All dose levels exceeded the maximum expected environmental concentration."

**REVIEWER'S COMMENTS:** An accurate determination of the level of toxicity of Dipel was not possible since an inadequate number of dosage levels were used within the dosage-mortality response range. It is not possible to determine the source of the observed toxicity since a sterile culture filtrate was not tested.

The tests used to analyze the experimental data were appropriate. The inability to calculate a valid $EC_{50}$ value was due to only two points being within the dosage-mortality response range.

In addition to the above summary statement by the Study Director (11), he states on page 14 "These effects were not considered to be due to the active ingredient of the compound. Correspondence with Abbott Laboratories revealed that the technical material was a formulation of *Bacillus thuringiensis* (Bt) solids and solubles. All levels showed an inert precipitate and some daphnids were observed coated with a particulate. Therefore, it is our opinion that all observed effects were attributed to the high solids concentration. These solids could have interfered with the daphnids filtering system thereby affecting growth, reproduction and survival." Since daphnids are filter feeders such is possible, but there is no experimental evidence to support that position. A study director should base his/her conclusions on study data, not on communications with the sponsoring company. If insufficient data exists to explain a result, further studies should be conducted. In another daphnid study to support the request for registration of Abbott Laboratory's *B. thuringiensis* subsp. *aizawai* product XenTari, similar effects were observed. The results of additional daphnid studies conducted with XenTari technical powder and fermentation beer suggested that the deleterious effects were due to a heat labile exotoxin complexed to the technical material during spray drying, not particulates contained in the test material. Since the *B. thuringiensis* isolate that Dipel is based on, HD-1, also produces heat labile exotoxin(s), it is probable that the results observed in this study were also due to the same or similar toxin.

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**Chemical:** Bacillus thuringiensis subsp. kurstaki

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